Advances in the Analysis of Hepatitis C Virus Specific T Cell Responses

Xinjian Zhang[#,1], Jun Dou[2] and Markus W. Germann*[,1]

[1]*Department of Chemistry, Georgia State University, Atlanta, Georgia 30302, USA*

[2]*Department of Pathogenic Biology and Immunology, School of Medicine, Southeast University, Nanjing, 210009, China*

Abstract: There is a growing consensus that cellular immune responses are associated with the clinical outcome of hepatitis C virus infection (HCV). The development of Tetramer staining, ELISpot, flow cytometry and epitope mapping technologies makes it possible to enumerate, phenotype and assess the proliferation and function of HCV specific T cells; as well as map and predict T cell epitopes and track the evolution of T cell epitopes. Such information is essential for the development of effective therapeutic and prophylactic HCV vaccines. This article summarizes the technical advances relevant to HCV specific T cell responses.

Keywords: Hepatitis C Virus (HCV), T cell, proliferation, tetramer, ELISpot, epitope mapping.

1. INTRODUCTION

HCV is a single stranded 9.7 kb, hepatotropic, RNA virus that belongs to the *Flaviviridae* virus family [1]. It has infected approximately 170-200 million people worldwide [2, 3]. Once infected, 15-25% individuals are able to clear the virus without treatment. The majority of infected individuals go on to develop chronic Hepatitis C [4] and approximately 5% die of complications from the chronic infection or from liver cancer. In the U.S., HCV infection is the most common indication for liver transplantation [5]. The underlying mechanisms responsible for the different outcomes are not well understood. In general, host immune responses, especially the cellular immune responses, play an important role in viral clearance, liver injury and persistent HCV infection. Further insight into the immune responses are crucial for elucidating HCV pathological mechanisms and ultimately for developing HCV vaccines [6-8].

The outcome of HCV infection is determined by viral factors and the host immune response, which includes innate and adaptive immune responses [9]. A vigorous peripheral and intrahepatic, $CD4^+$and $CD8^+$ T cell response against both structural and nonstructural proteins are main factors for virus clearance and resolution in self–limited HCV infections [4, 8-10]. In contrast, weak and narrowed epitope specific T helper cell (Th) and cytotoxic T lymphocyte (CTL) responses are associated with viral mutation and persistent infection [11-15]. Although the cellular immune response has been studied extensively, many questions remain unanswered. Fortunately, sensitive and reliable technologies have been developed that has made it possible to:

(1) count the number of HCV antigen specific $CD4^+$ T helper cells and $CD8^+$ cytotoxic T lymphocyte in peripheral blood and in the liver;

(2) analyze their proliferation and differentiation;

(3) determine their phenotype (effector and memory $CD4^+$/$CD8^+$ T cells);

(4) assess their specific function and ability to secret cytokines;

(5) map and tract T cell epitope evolution during the course of an infection.

These technologies have substantially improved our understanding of the HCV specific T cell responses, encompassing the kinetics, strength, specificity, diversity and breadth of HCV epitope(s).

2. T CELL PROLIFERATION ASSAYS

Following activation by HCV antigens, one of the first immune responses in an acute HCV infection is the vigorous proliferation of lymphocytes [16]. Because of its sensitivity (small number of cells required, $5x10^4$ cells/well) and high throughput, proliferation was conventionally measured with a ^{3}H-thymidine incorporation assay [17]. A limitation is that the negative control (T cell proliferation without HCV antigen stimulation) can be misleading in that a low negative control results in a false increase of the stimulation index, and vise versa. The flow cytometry based assays such as the carboxyfluorescein diacetate succinimidyl ester (CFSE) and bromodeoxyuridine (BrdU) based proliferation assays that also discern proliferations from different cell population ($CD4^+$ and $CD8^+$ T cells) [18] do not suffer from this problem. Incorporation of CFSE or BrdU into newly synthesized DNA during T cell activation is measured using anti-CFSE or anti-BrdU antibodies [19, 20].

*Address correspondence to this author at the Department of Chemistry, Georgia State University, Atlanta, Georgia 30302, USA; Tel: 404 413 5561; Fax: 404 413 5505; E-mail: mwg@gsu.edu

[#]Current address: Division of HIV/AIDS prevention (DHAP), Centers for diseases Control and Prevention (CDC), Atlanta, Georgia 30333, USA.

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3. ELISPOT AND INTRACELLULAR CYTOKINE STAINING(ICS)

Following proliferation, activated memory $CD8^+/CD4^+$ T cells inhibit viral replication by secreting antiviral cytokines such as interferon γ (IFN-γ) or tumor necrosis factor α (TNF-α) [21]. There are two methods for analyzing the effector function of HCV specific $CD4^+/CD8^+$ cells based on the secretion of cytokines: A) enzyme-linked immunosorbent spot (ELISpot) assay [22] and B) intracellular cytokine staining [23, 24]. These methods are based on the fact that HCV-specific memory or effector $CD4^+/CD8^+$ T cells, which were primed *in vivo* by HCV antigens, generate cytokines in just a few hours when they are stimulated again.

ICS is used for the quantitative detection of cytokine-producing antigen-specific T cells. In this assay, memory or effector $CD4^+/CD8^+$ T cells are challenged again by the cognate antigen and protein transport inhibitors (Brefeldin A or Monensin) are added to the culture medium to inhibit secretion. The cytoplasmatic cytokines are then captured by second antibodies. Using this method, the HCV-specific effector $CD8^+$ T cells with high levels of PD-1 but low levels of CD127 expression were characterized as an exhausted phenotype [25].

ICS is generally not sensitive enough to capture low frequency HCV specific $CD4^+/CD8^+$ T cells *in vitro*, but is useful for determining HCV antigen variation in specific T-cell lines during infection [13].

ELISpot uses cytokine specific immobilized antibodies (Fig. **1**). Cells of interest are plated and stimulated with a HCV specific antigen and the secreted cytokine is captured from the secreting cell. After washing, a biotinylated antibody specific for a different epitope of the detected cytokine is added and visualized [22]. Following detection, each signal (spot) represents one of the antigen specific T cells. The ELISpot assay has a high sensitivity and can be automated producing stable results even when cryopreserved peripheral blood monocytes (PBMCs) are used. Furthermore, ELISpot directly detects the frequency of IFN-γ secreting Th/CTL cells without *in vitro* expansion. A limitation of ELISpot is that it cannot be used to measure highly activated CTL populations (stunned phenotype of CTL), which temporarily fail to secrete IFN- γ [26-28].

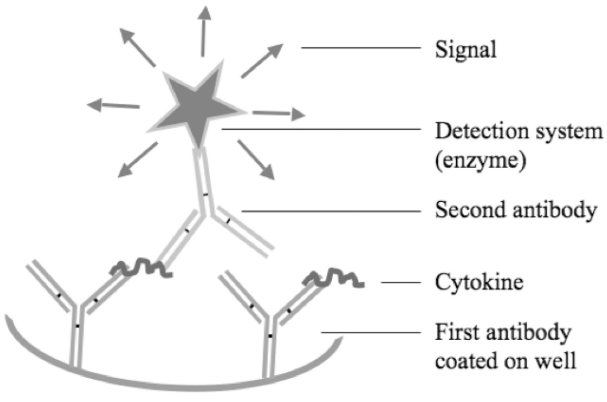

Fig. (1). The ELISpot assay uses cytokine specific immobilized antibodies that are coated onto the wells of a microplate. The secreted cytokine is captured from the secreting cell and visualized using streptavidin-horseradish peroxidase

4. CYTOTOXICITY T LYMPHOCYTE (CTL) ASSAYS

Following their introduction in 1968, cytotoxicity and limiting dilution assays [29] have been used extensively as antigen specific $CD8^+$T lymphocyte assays and have resulted in the identification of numerous HCV antigen epitopes [30-32]. For these experiments, traditionally, autologous B cells are isolated and immortalized with Epstein Barr virus and labeled with ^{51}Cr [32]. For the effector cell (HCV specific $CD8^+$ CTL) preparation, antigen specific T cells in PBMC or liver infiltrating lymphocytes (LILs) are isolated, expanded *in vitro*, and stimulated with IL-2, IL-7, or IL-12. At the final step, the labeled target cells are pulsed with HCV specific antigens. Both effector and target cells are then incubated together for 6 hours. The specific release of ^{51}Cr from the target cells is measured where lysis, and ^{51}Cr release from the target cell is mediated by perforin and granzymes. Perforin induces a pole in the target cell membranes when in contact with an effector cell. This allows serine proteases (granzymes) to enter the target cell, which in turn activates caspases resulting in apoptosis [33, 34]. As newer technologies emerged, the limitations of this standard method became apparent. First, a large number of PBMCs or LILs (from 1000 to million) are required for just a single HCV epitope evaluation. Second, the CTL response in HCV infected subjects is often multi-specific and polyclonal [30, 31]. Also, the major histocompatibility complex (MHC) is polymorphic; therefore, the evaluation of the various HCV epitopes in the context of different MHC molecules requires huge numbers of PBMCs/LILs [35-37]. These factors make it difficult to analyze all HCV epitopes in the context of each human lymphocyte antigen (HLA) alleleic variant in a single subject by this method. Another limitation is that the CTL assay requires cell culture to expand the freshly isolated PBMCs or LILs since such cells are frequently insufficient for performing the assay directly. Therefore, the cytotoxic activity that is observed represents the killing activity of selectively *in vitro* expanded HCV specific $CD8^+$ T cell lines from their precursors rather than the specific cytolytic activity of $CD8^+$ CTLs from the original blood or liver specimens. During cell culture and after exerting their effector function, some of the CTL will die from apoptosis [38]. Also, in some persistent infections, lytic cytotoxicity of HCV specific $CD8^+$ CTL is too weak or lost temporarily and cannot be measured [39-42]. This may provide a rationale why the specific cytotoxicity of $CD8^+$ CTL does not correlate with the clinical outcome of an HCV infection.

Even though the ^{51}Cr release assay for cytotoxicity is extensively used, several non-radioactive CTL assays have been developed recently. An example is the CFSE/PKH or FATAL assays, in which T cell mediated cytotoxicity is measured by flow cytometry or fluorescence microscopy [43-45]. These methods exploit the activation of caspases in the target cells by granzymes. In the flow cytometry CTL assays, target cells are labeled with peptides containing two fluorophores and a caspase cleavage site. In the uncleaved substrates, the emission of fluorescence is quenched by the

formation of an intramolecular complex of the two fluorophores. Upon caspase activation after the CTLs and target cell interact, the fluorophores are released.

The advantage of flow cytometry CTL assays is that it provides an early measure of CTL-mediated apoptosis [43]. The limitation of the granzyme and caspase based assays is that cell death may also occur without caspase or granzyme involvement [44, 45]. To remedy this, target cells are transfected with green fluorescent protein which is released after antigen specific $CD8^+$ T cell induced cytolysis. This method has the same features as the conventional ^{51}Cr release assay but is 8 to 33 fold more sensitive [46].

For rapid and sensitive detection of the cytotoxicity of $CD8^+$ T cells, Perkin-Elmer Life Science developed a kit based on fluorescent lanthanide chelate labels. After cytolysis, the lanthanide is released from the target cells within minutes. In the presence of a low pH enhancer solution, free lanthanides (Eu^{3+}, Sm^{3+}) form a highly fluorescent and stable chelate [47]. An alternative method is to measure membrane proteins CD107a/b on $CD8^+$ T-cells by flow cytometry [48]. These proteins are mobilized to the cell surface during degranulation which is directly associated with cytolytic activity of effector $CD8^+$ T cells [48]. To elucidate the functional and full phenotypic characteristics of the responding CTLs, combined methods are needed.

In summary, from the perspective of target and effector cells, *in vitro* CTL assays can be divided into two categories: Direct measurement of cytotoxicity based on flow cytometry CTL, caspase assay or ^{51}Cr release from target cells; and indirect CTL assays based on the detection of specific molecules which are used by $CD8^+$ T-cells to lyse target cells (flow cytometric CD107a/b degranulation assay, perforin/granzyme ELISpot assay).

5. TETRAMER STAINING FOR ENUMERATING AND PHENOTYPING HCV SPECIFIC T CELLS

The preparation of peptide-MHC tetramers has enabled immunologists to accurately enumerate and trace antigen specific $CD4^+$/$CD8^+$T lymphocytes both in peripheral blood and in the liver. This has revolutionized our understanding of cellular immunology in viral infection, tissue transplantation and tumors [49, 50]. To appreciate the relevance of the peptide-MHC tetramer approach, antigen processing/presenting during HCV infections will be highlighted. HCV proteins present in hepatocytes, Kupffer, sinusoidal, endothelial and dendritic cells are degraded to peptide epitopes. The viral peptide epitopes are then transported to the rough endoplasmic reticulum and assemble with β_2 microglobulin to form the peptide-MHC I complexes that are presented to the circulating CD8+ T cells [51]. The antigen-specificity of a T-cell line/clone is determined by the T cell receptor (TCR) which recognizes the peptide-MHC complex. A construct of a tetrameric peptide-MHC complex greatly increases the binding affinity for the TCRs (Fig. **2**). Therefore, fluorescence-labeled peptide-MHC tetramers can be used to screen PBMC or LIL samples to detect antigen-specific $CD8^+$ T cells by flow cytometry. If MHC class II molecules and $CD4^+$ peptide epitopes are employed, the resulting tetramers are suitable for tracing Th cells. Numerous reports have been published on the determination of HCV specific $CD4^+$ Th and $CD8^+$ CTL epitopes using a combination of tetramer staining, flow cytometry and ELISpot assays [52-56]. For example, if an IFN-γ monoclonal antibody is added in multicolor flow cytometry, it becomes possible to analyze the IFN-γ secreting function status of MHC I tetramer-$CD8^+$ CTL and MHC II tetramer-$CD4^+$ Th cells by ELISpot assay [22]. If CD45RA and CD27/CD28 monoclonal antibodies are available, the activation phenotype of $CD45RA^+CD27^-CD28^-$ and the memory phenotype of $CD45RA^-CD27^+CD28^+$ of the $CD8^+$ CTL and $CD4^+$ helper T cell can be determined simultaneously by tetramer staining in conjunction with multicolor flow cytometry [57, 58].

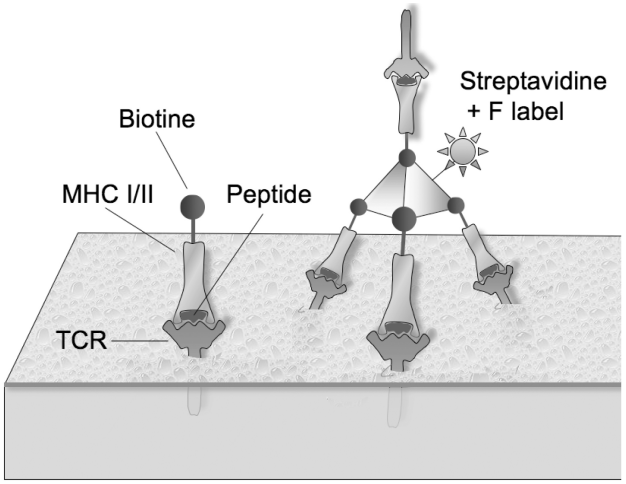

Fig. (2). Schematics of the interaction of MHC Monomer and Tetramer with TCR. MHC I heavy and light chain are refolded with a specific antigen peptide and biotinylated. Tetramers are formed by mixing the MHC-peptide monomers with fluorescently labeled streptavidin. If MHC-I and MHC-I restricted HCV antigen peptides are used, the tetramer binds $CD8^+$ T cells. For MHC-II and corresponding antigen peptide, the tetramer is specific for $CD4^+$ T cells.

The tetramer staining assay is a single cell-based method, which can be directly employed to determine the frequency of antigen specific $CD8^+$/$CD4^+$ T cells without the need for *in vitro* cell culture or expansion of the PBMCs and LILs. Therefore, the assay results (obtained by using fresh PBMCs and LILs) reflect the real frequency of HCV antigen specific $CD8^+$/$CD4^+$ T lymphocytes. If the frequencies of tetramer positive T cells are low, it is possible to enrich the cells using magnetic beads (containing CD4 or CD8 antibodies). Recently, a novel strategy was developed for rapid tetramer generation that is based on the production of correctly oxidized and biotinylated MHC I heavy chain in *E.coli*. The readily isolated functionalized proteins can then be refolded, if necessary, and with the addition of streptavidin form the desired tetramers. It represents a simple and versatile technique that is amenable for scale up for the production of many peptide-MHC tetramers [59]. If multiple peptide-MHC tetramers are combined with a high-throughput screening, it is possible to screen peptide-HLA binding covering the whole HCV proteome [60]. A requirement is that both the T cell epitopes and the HLA typing (MHC I/MHC II) of the

infected patients must be known in advance for the preparation of the MHC I/MHC II tetramers.

6. HLA/MHC ALLOTYPE AND HAPLOTYPE DETECTION ASSAY

The major histocompatibility complex (MHC) located on the short arm of chromosome 6, encodes the human leukocyte antigen (HLA) class I and II genes. The protein products (MHC I/MHC II) play an essential role in the adaptive immune response. HLA/MHC allotype and haplotype typing determine surface antigens on lymphocytes from HCV infected patients. The peptide binding region polymorphism of the MHC determines antigen specificities and strength of the immune response. Therefore, HLA allelotype and haplotype may determine the outcome of T cell response and HCV infection by influencing the strength of the T-cell response and breadth of the T-cell receptor repertoire [61].

The traditional method of HLA typing is the "lymphocytotoxicity test". Briefly, freshly isolated PBMCs are mixed with a specific HLA antibody, and fresh rabbit serum is added as the complement source. If the PBMCs contain the specific antigen corresponding to the added HLA antibody, the cell bound antibody activates the complement system, resulting in cell lysis. In presence of the DNA intercalator ethidìum bromide lysed cells appear red, while the permeable dye acridine orange stains live cells green. Several DNA based methods have been developed for HLA typing, including hybridization with sequence-specific oligonucleotide probes and polymerase chain reaction (PCR) amplification with sequence-specific primers (PCR-SSP) [62, 63]. In the PCR reactions, a completely matched primer is used more efficiently than a primer with one or more mismatches which results in more PCR products for the matched HLA specific primer. The determination of alleles (HLA-antigen) is then based on the presence or absence of amplified PCR products [63, 64]. HLA typing has provided important information for investigating the adaptation of circulating viruses to HLA class I-associated selection pressure at population level. For example, comparison of HCV CTL epitope sequences from Asia and Europe suggests that the frequency of the HLA-A*01 allele in a population may influence the frequency of the HCV CTL escape variant in circulating strains. In HLA-A*01-rich populations the majority of HCV isolates is adapted to immune pressure [65-67]. Also, HLA typing is necessary to identify and map MHC-restricted T-cell epitopes, which are potential immunogens for T-cell based vaccine design.

In order to translate the experimental results from animal models, HLA transgenic mice have been produced and used to identify T cell epitopes in HCV proteins [68]. More recently, human $CD34^+$ cells (which have the potential to differentiate into $CD4^+$/$CD8^+$ T cells) and human hepatocyte engrafted mice were established to evaluate HCV specific T cell response elicited by HCV vaccines [69, 70]. Marodon and colleagues have demonstrated that the humanized (non-obese diabetic)/(severe combined immunodeficiency) NOD/SCID mice engrafted with human $CD34^+$ cells mounted a HCV specific but weak HLA-restricted cellular immune response after immunization with HCV envelope glycoproteins [69]. Also, adult human hepatocytes can be repopulated (10%) in liver tissue of the NOD/SCID mice [70]. There are however, several limitations of human HLA transgenic and humanized NOD/SCID mice models:

1. Some T cell epitopes are processed properly in humanized HLA transgenic mice, but not presented (cross-species incompatibility in antigen-processing and presentation). The mechanism of antigen-processing and presentation in humanized mice is different from humans [71].
2. Male mice suffer various autoimmune like symptoms since estrogens affect $CD4^+CD25^+Foxp3^+$ regulated T cell development.
3. The repopulation efficiency of the human hepatocytes in humanized mice is low and needs to be improved.

7. MAPPING T CELL EPITOPES

7.1. T Cell Epitope Prediction and Screening

Prediction of T cell epitopes is pivotal in characterizing the immune response for intracellular pathogens (hepatitis B virus (HBV), HCV, human immunodeficiency virus (HIV) and mycobacteria). The prediction is based on sequence data of individual and pool peptides eluted from MHC-peptide complexes [72], crystal structures, and positional scanning of synthetic combinatorial libraries (PS-SCL) [72, 73]. Falk and colleagues extracted and sequenced MHC associated peptides from infected cells [74]. The following features were observed: a specific MHC class I molecule may bind an individual peptide or a mixture; the peptides are typically 8 to 10 amino acids in length and bear key amino acids at specific anchor positions.

The binding motifs are allele specific, for example, the HLA-A2 binds a 9 residue peptide, where Leu and Val are anchor amino acids at positions 2 and 9. There are some commonalities. All epitopes appear to have a hydrophobic C terminus, while the N terminus is highly variable. The program Epimatrix [75] was developed to predict binding to a specific MHC molecule. Based on the prediction, a score is computed for the relative binding enhancement/inhibition contribution of each amino acid at a particular position. By comparing the scores of putative to known binders and non-binders, the probability of each peptide fragment as epitope will be predicted according to the estimated binding potential.

Another approach to predict T cell epitopes is PS-SCL . Here, each peptide residue is taken into consideration, in contrast to Epimatrix, which solely focuses on anchor residues. PS-SCL is based on the assumption that each position is independent and additive. Each virtual library consists of a complex mixture of peptides, all of which have one position fixed while all other positions are randomized to the 20 amino acids in equal proportion resulting in a total of 200 combinatorial libraries.

The results of the T cell proliferation response are arrayed into a score matrix (20 aa x 10 positions). Each fixed amino acid at a certain position generates a stimulation index (SI):

$$SI_{ij} = \frac{\text{(Mij mean proliferation response to (I, J)-th mixture)}}{\text{B mean of the background (no peptides).}}$$

In this way, the best-matched amino acid (with the highest score) will be defined. These methods allow the prediction of potential peptide epitopes from any protein [76-79].

A limitation is that these methods only estimate the binding capacity of a peptide. Although binding of a peptide is required, it is not sufficient for T cell activation. Furthermore, MHC independent pathways may exist for T cell activation. Therefore, the predicted T cell epitopes require experimental confirmation. Lauer *et al.* developed a high resolution ELISpot method to screen T cell epitopes. This method identifies minimal optimal epitopes in a peptide pool with overlapping HCV sequences (OLP) [80]. OLP are short synthetic peptides of 8-20 amino acids based on HCV proteins. Combined with semiautomated ELISpot and CTL, OLP has been successfully used for identifying minimal and optimal HCV specific $CD4^+/CD8^+$ T cell epitopes [68,81-82]. This combination of techniques enables the identification of potential HCV T cell epitopes covering the breadth of complete $CD4^+/CD8^+$ T-cell responses in the context of all autologous HLA alleles and simultaneously monitoring the generation of several cytokines [82].

7.2. Validation of the Potentional T Cell Epitopes

Since high scores obtained from computer programs do not necessarily signify that the epitopes have a high affinity for MHC molecules, there are 2 approaches commonly used for experimental validation. One is a competitive MHC binding assay, where the epitope under study and a radio-labeled standard peptide are added to soluble MHC I. The binding ratio (peptide/radio-labeled standard) is employed to evaluate the binding to a particular MHC molecule [83, 84]. The other utilizes transporters associated antigen processing (TAP)–deficient cell lines and T cells. TAP deficient cells lack the antigen processing ability, consequently this system measures the binding of exogenously added peptides. For example, in the T2B7 cell line the peptide binding affinity to MHC–B7 can be assessed, while binding to another MHC molecule requires a different cell line with the corresponding MHC. Flow cytometry can evaluate the MHC binding affinity of the peptide using biotinylated peptides and avidin-FITC conjugates [85, 86].

Following the validation of the T cells epitopes by the MHC molecule-binding assay *in vitro*, their T cell response capacities can be further evaluated *in vivo* using synthetic peptides of the T epitope vaccine candidates. In immunization assays, human MHC I/II molecule transgenic mice are required for the *in vivo* T cell activation experiments [71].

Table 1. Summary of the Advantages and Issues Associated with Each Method for Characterization of HCV Specific T Cell Responses

Method	**Advantages and Limitations**
ELISpot assay	•First option to explore HCV specific T cell responses after vaccination and clinical intervention •Does not require *in vitro* cell expansion. •Simultaneous screening of T cell epitopes and mapping of T cell responses spanning entire HCV protome in the context of all HLA alleles for multiple cytokines. •Cannot be used to measure the highly activated CTL populations which temporarily fail to secrete IFN-γ[27–29].
Proliferation assay	•Second most popular method to measure the immune responses during vaccine and clinical trials. •Together with cell depletion it can be used to determine predominant responding cell populations. •Not suitable to assess the T cell function at the single cell level. •Proliferation is one of several aspects of T cell function. Combined with cytokine secreting assays is required to generate T cells functional profiles in chronic HCV infection [34].
Intracellular cytokine staining (ICS)	•It is not sensitive enough to detect low frequency HCV-specific T-cells *in vitro* in chronic HCV infection.
TCR based peptide–MHC tetramer staining	•Overnight resting thawed PBMC prior to measurement may increase the detection sensitivity. •Most important method for direct phenotype analysis of the HCV specific T cells. •Tetramer positive HCV specific T cells may not have the potential to secrete cytokine (IFN-γ). •The frequency of the PBMCs that can be stained *in vitro* is usually below the detection limit (HCV specific T cells in PBMCs can be enriched with magnetic beads).
CTL assays	•High sensitivity especially when T cell lines are used. •Contributed to the identification of many $CD8^+$ T cell epitopes. •CTL positive $CD8^+$ T cells have the capacity to lyse target cells expressing HCV antigens. •It requires *in vitro* cell expansion, some subsets of T cells may not expand well or at all and are underrepresented. •Some subsets of $CD8^+$ T cells (stunned phenotype) are lacking cytolytic activity, this phenotype of $CD8^+$ T cells can not measured by CTL assay.

Several candidate T cell epitopes vaccines were shown not only to activate the antigen specific $CD4^+$/ $CD8^+$T cells but also to induce cross-recognized T cell responses. This cross-recognized T cells response was able to protect chimpanzees from a heterogeneous virus challenge [87-89].

8. CONCLUSION

This article describes technological advances, summarized in Table **1**, that enable evaluation of different aspects of the HCV cellular immune response. For example, T cell proliferation and effector function assays (CTL, ELISpot) measure HCV primed T cells at different stages of activation. T cell epitope mapping, ELISpot and CTL assays may work together to identify protective T cell epitopes from self-resolved HCV infected subjects. Despite considerable progress, additional developments are needed to shed light on 1) protective T cell immunity; 2) identification of phenotypic markers to define memory T cell subpopulations; 3) phenotypic, number, and functional analyses of HCV specific $CD4^+$/$CD8^+$ T cells in the liver at different disease stages, especially at the early stages; and 4) a consensus for assay standardization/validation and statistical analysis. Hopefully, ELISpot, Tetramer-flow cytometry, new CTL assays and HCV sequence variation analyses can work together to further our understanding why HCV can escape immune responses so that successful strategies for vaccine development can be developed.

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ABBREVIATIONS

BrdU = Bromodeoxyuridine

CFSE = Carboxyfluorescein diacetate succinimidyl ester

CTL = Cytotoxic T lymphocyte

ELISpot = Enzyme-linked immunosorbent spot

FITC = Fluorescein isothiocyanate

HBV = Hepatitis B virus

HCV = Hepatitis C virus

HLA = Human lymphocyte antigen

HIV = Human immunodeficiency virus

ICS = Intracellular cytokines staining

IFN-γ = Interferon gamma

LILs = Liver infiltrating lymphocytes

MHC = Major histocompatibility complex

NOD/SCID = Non-obese diabetic/severe combined immunodeficiency

OLP = Overlap peptide

PBMC = Peripheral blood monocyte

PCR = Polymerase chain reaction

PS-SCL = Positional scanning of synthetic combinatorial libraries

SI = Stimulation index

TAP = Transporter associated with antigen processing

TCR = T cell receptor

Th = T helper cells

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